University of Wisconsin Library

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the University of Wisconsin Library are open for inspection, but are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages may be copied only with the permission of the authors, and proper credit must be given in subsequent written or published work. Extensive copying or publication of the thesis in whole or in part requires also the consent of the Dean of the Graduate School of the University of Wisconsin.

This thesis by..... has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

A Library which borrows this thesis for use by its patrons is expected to secure the signature of each user.

NAME AND ADDRESS	DATE

A DAMPING-OFF FUNGUS OF RADISHES

BY

DAVID RICHMOND SANDS

Thesis Submitted for the Degree of

MASTER OF SCIENCE

UNIVERSITY OF WISCONSIN

1922

Contents.

A DAMPING-OFF FUNGUS OF RADISHES.

Introduction.

During the month of June 1921, radishes of the White Icicle variety showing blackened areas were brought to the Department of Plant Pathology from a few gardens in Madison. In general appearance these radishes were very similar to those which Edson[8] had formerly described as being affected with an organism which he named Rheosporangium aphanidermatus. It was with these radishes that the writer began to make a study of the disease to see if it was produced by the same or other organisms.

Literature Reviewed.

In 1912 J. T. Barrett[1] described a root disease of radishes as being serious in many localities in Illinois and that sometimes a large part of the crop was rendered unmarketable. He states that the causal organism is Aphanomyces laevis de Bary, one of the Saprolegniaceae. It causes a peculiar browning or blackening of a portion or all of the root. Seedlings under certain conditions are also affected. The stems become shrunken at the surface of the ground and the plants finally fall over. The mycelium is very delicate, intercellular and partially dissolves the middle lamella of the

cell walls. Swarm spores are produced in large numbers and oospores very probably carry the fungus over winter.

In 1913 H. A. Edson[6] published an article on the damping-off and root rot parasites of sugar beets. He states that four fungi have been shown to stand in causal relation to seedling troubles. These are Pythium de Baryanum Hesse, Aphanomyces laevis de Bary, Phoma betae Frank, and a species of Rhizoctonia probably identical with the form described as Corticium vagum B & C var. solani. Burt. though the perfect stage has not been observed.

During the following year Miss Willis[11] made a study of the disease causing the black root of radishes and gave an account of it in her M.S. thesis, 1914. She found the disease on red radishes, where it caused a purple discoloration of the radish root. As the infection was very much similar to that on white radishes sent to the Department of Plant Pathology by Professor Barrett in 1912, Professor L. R. Jones suggested the possibility of it being due to the same organism, viz. Aphanomyces laevis de Bary. The organism was isolated and proved to be the same as that which Edson had isolated from sugar beets and which he thought was Aphanomyces laevis - asexual reproduction being by means of zoosporangia which produced motile biciliate zoospores and sexual reproduction by means of oogonia and antheridia. She describes the formation of zoospores as follows: "The swarm spores of the black root organism are formed from protoplasm which bursts from the zoosporangia, which are thickened irregular portions of repro-

duction branches on thread-like mycelium." On account of the shape of the zoosporangia and the formation of zoospores she does not consider that it is Aphanomyces laevis de Bary, and suggests that it might belong to a different species or possibly to another genus.

In 1915 H. A. Edson[7] described some of the seedling diseases of sugar beets and stated that the fungus originally reported as Aphanomyces laevis de Bary had been found to be a new one. In reference to this organism he writes as follows: "Through the courtesy of the Kaiserliche Biologische Anstalt at Dahlem, Germany, and Dr. Leo Peters, of that institution, the author was permitted to isolate Aphanomyces from the experimental fields of the Anstalt. An organism was secured from damped-off seedlings, which Dr. Peters identified as the organism with which he had worked and which conformed in every respect to de Bary's description of A. laevis ..... . The morphological studies, however, prove that the American fungus with which we have been working is not A. Laevis but a hitherto undescribed organism." He further states, "The disease produced by this fungus is so similar to that reported by Barrett that they are not readily distinguishable and it may be that either of these organisms is responsible for the disease in any of the stations mentioned." The stations referred to are the various places where black root of radish has been observed.

In July of the same year Edson[8] published a more detailed account of the organism and named it Rheosporangium

aphanidermatus. He states that protoplasmic streaming leads to an accumulation of material in the extremities of hyphae which become enlarged and somewhat distorted. A partition wall is laid down cutting off the swollen portion which varies greatly in size and shape and which he calls the presporangium. This presporangium enlarges and finally ruptures. The contents then flow out into a thin membranous structure and the mass takes a spherical form. This body, the zoosporangium, remains at the mouth of the presporangium wall during the cleavage of the contents into zoospores which are liberated by the rupture of the wall. He places the fungus in the Saprolegniaceae and follows Mindens classification. As the formation of the presporangium and the egress of zoospores is different from any mentioned by Minden he has considered it advisable to place it in a new genus.

In 1921 C. W. Carpenter(5) published an account on morphological studies of the pythium-like fungi associated with root rot in Hawaii. He states that the cane fungus agrees very closely in both the asexual and the sexual stages with the sugar beet root rot fungus Rheosporangium aphanidermatus first described by Edson. In reference to the asexual stage he says the presporangia vary greatly in size and shape but they generally consist of several closely attached spherical masses, densely and finely granular and slightly brownish in color. The portions are characteristically more globose than represented by Edson for Rheosporangium aphanidermatus. He considers the cane fungus, morphologically the same as Rheosporangium

aphanidermatus and Pythium butleri.

Symptoms.

During the seedling stage the disease, when severe, causes a damping-off of the plants. The stems at the surface of the ground become shrunken and somewhat blackened and the plant falls over. In less severe cases the cortical portion may be affected and a long spindly root produced. In other cases the plants develop as usual, that is, in so far as can be noticed from the above ground parts, but when pulled diseased areas are found upon the roots. These areas are brown to black and in many cases involve a considerable portion of the root. Considerable deformity often occurs, due to retardation of growth. In early stages the affected tissue remains sound a and there is no unpleasant taste or odor. In later stages, the cells may become broken down and rot develops.

It is possible, however, that the blackened areas may be partly due to other organisms. The organism under discussion has been isolated from the blackened area as mentioned elsewhere but not under conditions to prove that it is the only one causing the trouble.

Experiments in the field.

In one of the gardens from which the infected radishes were brought to the department, the greater part of the crop was unfit for use. In another garden over 50% in one row were

infected, whereas in another row about twenty-five feet away none were infected.

In the former garden, sugar beets, turnips, cabbage and three varieties of radishes were planted about the twentieth of June, in the same place as where the diseased radishes had been grown. In the latter garden a row was planted partly sugar beets and partly radishes (White Icicle). The object of planting the sugar beets, turnips and cabbage was to see if any of these were susceptible to the disease.

The weather for a month or more was very dry and hot and although both gardens were well watered on two occasions yet growth was slow and no damping off or other symptoms of disease could be noticed.

When the radishes were pulled only two were affected and one of these only very slightly. Soil was later brought into the greenhouse from one of the gardens and experiments carried on there during the fall and winter.

Experiments in the Greenhouse.

In the greenhouse experiments, sugar beets and White Icicle radishes only were used. In addition to several pots which were used in the experiments, two boxes were filled with soil and planted. In one of these boxes soil from the garden only was used, while in the other about one part of the diseased garden soil was mixed with three parts of the usual greenhouse soil. In the box containing soil all of which came from

the garden, the radishes and sugar beets damped-off very fast and although several replantings were made yet very few came through the seedling stage and those that did usually had a long spindly root. In the box containing soil only about one-quarter of which came from the garden, there was some damping-off when radishes were about an inch high, whereas those that did not damp-off showed no signs of disease until pulled about two months later when a number of them were affected with the disease.

Two infected radishes were placed in each of three flower pots containing soil from the greenhouse and allowed to decay. Radish seeds (White Icicle) were then planted in these pots, the pots set outside and watered regularly. In about two months the radishes were pulled but none showed any signs of black root.

Isolation of Organism.

Various attempts were made to isolate the organism directly from the blackened areas of the radishes that were pulled from the gardens in June.

Radishes were immersed in bichloride of mercury (1-1000) for three to five min., rinsed in sterile water then the outer surface removed under sterile conditions and pieces of the interior plated out on string bean, oatmeal and potato agar. - but in no case did any organism develop.

Soil was sterilized in the antoclane for two hours at 15 lbs. Infected radishes were mashed up and mixed with this

soil. Radish and sugar beet seeds were planted and left for several weeks, but no infected plants were produced. This experiment was tried only once, however, as the infected radishes had all been destroyed before an attempt was made to repeat it.

Sugar beet seeds were planted in some of the unsterilized soil from the garden where diseased radishes had grown. When up a short distance the seedlings began to damp off. Some were sterlized in bichloride of mercury (1-1000) for one min., rinsed in sterile water and plated out on string bean agar and in twenty-four hours a fungus growth had developed.

Sterilized soil was inoculated with this organism and planted with sugar beets. Damping-off occurred as previously and the same organism was re-isolated.

Similar attempts were made to isolate the organism from damped-off radishes grown in unsterilized soil from the garden. When they were sterilized with bichloride of mercury, and plated out, no growth developed and when not sterilized bacteria over-run the plates. At length prune agar was tried and the same organism was obtained as that isolated from sugar beet seedlings.

Sterilized soil was inoculated with the organism and planted with radishes as had previously been done with sugar beets. When the damped-off radishes were plated out the organism was reisolated.

During the month of March a few pieces from one of the diseased radishes grown in the greenhouse were plated out on prune agar. In less than forty-eight hours, a mycelial growth had developed and this proved to be the same organism as isolat-

ed from other sources. This radish was grown in the box containing soil about one-quarter of which had come from the garden.

Similar attempts had been made during the previous summer to isolate the organism from the diseased areas but prune agar had not been used. When other media, such as string bean agar, were used without lactic acid being added, bacteria soon over-ran the plates and when lactic acid was added even in small quantities, no growth developed. Owing to the limited supply of diseased radishes, no further attempts to isolate the organism could be made at this time.

When prune agar has been used in attempts to isolate the organism from radishes grown in garden soil, other organisms have nearly always been present and some of these may, to a certain extent, be responsible for the severe damping-off which occurs in some cases. One organism frequently present consists of five septate mycelium, but as the one under discussion grows more rapidly, it soon passes beyond the region of this one.

Inoculation Experiments.

After isolating the organism, several radishes and sugar beets, while growing in the garden during the summer, were inoculated with the mycelium growing on string bean agar. All inoculations were negative, probably due to some extent to the hot dry weather.

At a later date, inoculation experiments were tried in the greenhouse. A few cans of soil were sterilized at fifteen lbs. for two hours, and planted with radish seeds (white icicle). When radishes were from one-half inch to

one inch in diameter, those in two cans were inoculated with media containing mycelium and those in another can inoculated with media only as a check.

In a few days the disease developed in about two-thirds of those inoculated with media, containing mycelium, whereas those inoculated with media only remained healthy, although left for a month before pulling. Some of those that were infected were later plated out and the organism reisolated.

This experiment was repeated later and the same results obtained. There was this difference, however, that where the disease developed, there was more softening of the tissues than is usual when plants become affected while growing in the garden.

In one of these cans containing radishes which had been inoculated, two that developed the disease were allowed to decay and the can was then replanted. During the seedling stage, no severe damping-off occurred, but many of the plants showed a slightly shrunken and somewhat darkened area just at the surface of the ground and when these were plated out on prune agar, the cycelium of the organism soon developed.

Other cans containing sterilized soil were inoculated with the organism at the time of planting, - string bean agar with the cycelium being used. Practically no damping-off occurred but when the radishes became a fair size and were pulled, one showed a slightly blackened area from which the organism was isolated.

Two cans containing soil were sterilized, - one was inoculated and planted with radish seeds, the other planted and left as a check. The organism used for inoculation in this case, was grown on oat meal agar for about two weeks; sterile water was then poured over the surface and the surface scraped with a platinum needle in order to obtain the organism free from agar. The water with contents was then poured over the soil. More than half the seedlings damped-off in the inoculated can, leaving a very thin stand, whereas those in the check showed no sign of the disease.

A similar experiment was tried with sugar beets. All the seedlings in the inoculated can damped-off soon after coming through the ground, whereas those in the can planted as a check remained healthy.

Identification?

After isolating the organism, a study of it was made to see if it was similar to Rheosporangium aphanidermatus. Petri dishes of string bean agar were inoculated and then small portions of this agar with the mycelium were placed in petri dishes containing sterile water and also in petri dishes containing sugar beet seedlings which had been sterilized in water (according to the method described by Edson [8]). Oogonia and antherdia developed in abundance in both cases, but no zoospores were obtained.

In order to make closer studies of the organism, hanging drops were made (van Tieghem cells). Mycelium from the string bean agar in sterile water and also mycelium from the

sugar beet seedlings in sterile water was transferred to these drops. The handing drop in some cases was sterile distilled water, in other cases, sterile top water or water from sterilized radishes or sugar beets. In all cases, oogonia and antheridia were fairly numerous, and when the mycelium came in contact with the glass or with the edge of the drop abnormal structures developed, but in no case could any presporangial stage be found either in petri dish cultures or in hanging drops. Hence, it was evident that the organism was not Rheosporangium aphanidermatus.

Morphology.

The mycelium consists of nonseptate hyphae which grow rapidly on suitable solid media, such as string bean or oat meal agar covering the surface of the medium on a four inch petri dish in about forty-eight hours, when at room temperature. It is much branched and frequently, when grown upon media, a few short branches develop quite closely together and become somewhat coiled to form gnarled or knotted masses. This is quite noticeable on prune agar. These branches are frequently slightly greater in diameter than the ordinary hyphae, but in no way do they appear to form a sporangium of any kind.

Streaming of protoplasm in the hyphae is quite noticeable either in petri dish cultures or where mycelium is growing in water in petri dishes or in hanging drops, and occurs after a period of twenty-four hours from the time of inoculation. This streaming may occur in either direction in the hypha but the movement is usually towards the distal portion. Where the

mycelium has been growing on sugar beet seedlings in water, the streaming has been observed taking place from the distal end towards the seedling and the moving protoplasm followed until the movement slowed down and the hypha lost among the mass around the seedling. In many cases the movement of the protoplasm arises in some part of a hypha, gains in rapidity for some distance and then gradually slows down until no further movement can be observed.

The movement of the protoplasm has also been observed coming in from a branch, passing towards the distal end of the hypha for some distance and then moving out into another branch. In many cases where such streaming of protoplasm has occurred, the outer parts of the hyphae have frequently been observed for some time, but no unusual development in growth has been noticed.

When a piece of medium with mycelium is transferred to a petri dish containing water, sexual organs are formed in abundance. Similar results are obtained by transferring some of the mycelium to vessels containing water with sugar beet seedlings either fresh or autoclaved, cabbage leaves, cooked potato and dead flies.

The oogonium develops terminally usually on a branch and when nearly full size, it is cut off by a cross wall from the supporting hypha. The antheridium arises from the hypha bearing the oogonium and close to it and forms a somewhat curved structure, the outer end which comes in contact with the oogonium being the larger. During the development of the antheri-

dium, the oogonium is usually somewhat crowded to one side so that in place of being terminal it has more or less of a lateral position.

Shortly before fertilization takes place, the oosphere (egg) forms within the oogonium and at this stage the entire contents of the egg is made up of rather large rounded masses and a narrow clear zone separates the egg from the ocgonial wall.

As the antheridium reaches maturity, it is cut off by a cross wall. As fertilization is about to take place, the part in contact with the oogonium develops a beak (fertilization tube) which penetrates the oogonial wall and comes in contact with the oosphere, the contents of the antheridium then pass through this into the oosphere. This portion is often not visible until after fertilization has taken place. From the time the antheridium starts to form until fertilization takes place, requires about six or seven hours.

After fertilization, the wall of the oospore becomes much thickened and the antheridial wall frequently remains attached for some time. In nearly all cases, there is only one antheridium for each oogonium and usually formed from the same hypha as the oogonium, but at times the antheridium may arise from an adjoining hypha. One case has been observed where the antheridium developed as usual from the same hypha as the oogonium and another antheridium developed from an adjacent hypha, but the latter did not appear to take part in the fertilization.

Sometimes structures that appear to be conidia develop either terminally or intercallary and during their develop-

ment these cannot be distinguished from oogonia but when mature no oosphere forms. These have frequently been observed in hanging drops and in a few cases have produced one or more germ tubes. The place of origin of these germ tubes is indefinite and they never extend to any length.

When the organism is grown on some forms of media such as string bean or prune various abnormal structures develop, but in no case has the formation of sporangia been observed.

Taxonomy.

From studies so far made, the organism under discussion seems to be a Pythium but not sufficient is as yet known to attempt to classify it, as neither the production of zoospores nor the germination of oospores has been observed.

In Butler's (3) classification of the genus Pythium, there are two sub-divisions, viz: Sub-genus Aphragmium - Sporangia filamentous etc, conidia unknown, and Sub-genus Sphaerosporangium - sporangia spherical, oval etc. (not filamentous). Under the latter sub-genus, there are two divisions - A. sporangia proliferous, Conidia unknown and B. sporangia not proliferous, often transformed into Conidia. Under division B there are three sections:

1. Oogonia and oospbres smooth.
2. Oogonia smooth and oospores spiny.
3. Oogonia spiny and oospores smooth.

As both oogonia and oospores of the organism are smooth, it probably belongs to the first section. This section includes seven species, among these there are three species whose oospore

does not fill the oogonium, viz:

P. de Baryanum	Hesse	-	Parasitic in seedlings, sporangia subspherical.
P. vexans	de Bary	-	Saprophytic in soil, sporangia rare, conidia irregular.
P. ultimum	Trow	-	Saprophytic, sporangia not known, conidia sub-spherical.

Miyake (9) in describing the fertilization of P. de Baryanum Hesse, states that about the same time that the oogonia are cut off, one or more antheridia begin to develop close to each oogonium. Frequently there are two antheridia and sometimes three, and these are of two kinds, w stalk antheridia and branch antheridia. Hence, the number of antheridia which develop does not agree with the number found in this organism, which is nearly always one, two being observed in rare cases.

In the case of P. vexans de Bary, one of the characteristic features is the attachment of the antheridium and the oogonium. The antheridium is closely applied to the oogonial wall and is somewhat fused with it, for a large part of its circumference. In general appearance, the two structures form a large pear-shaped cell divided into two parts, the oogonium comprising the larger portion.

In reference to P. ultimum Trow, a detailed description is given by Trow (10) who states that it is saprophytic. He says, "no zoospores have been seen at any time, and it may be regarded as certain that the conidia are no longer capable

of producing them for (1) there is no indication of a terminal beak such as is usual in sporangia (2) no germination takes place in distilled water (3) tube germination takes place although slowly and irregularly, on material placed in running top water, (4) in suitable nutrient solutions each conidium may give rise to four or five germ tubes whose place of origin is quite indefinite and (5) empty conidia have never been seen although numerous experiments have been made on germination."

In reference to Oospores, he says they have a smooth thick two-layered wall of yellowish color, enclosing finely granular cytoplasm, a central reserve globule and one lateral nucleus, germinating at once or after a period of rest extending to seven months and always by one or more germ tubes.

Since the organism under discussion develops as a parasite, and since the germination of oospores has not been observed nor germination of conidia except in a few doubtful cases, there is not sufficient evidence to place it in this species.

The size of the reproductive bodies of P. de Baryanum given by Butler (3) of P. ultimum given by Trow (10) and this organism, are as follows:

P. de Baryanum Hesse.

Conidia 15.0 to 25.0 u, round oval or irregular.

Oogonia 20.0 to 25.0 u, terminal and intercalary.

Oospores 14.0 to 18.0 u.

Sporangia not found in many cases.

P. ultimum Trow.

Mycelium 1.7 to 6.5 u.

Conidia 12.0 to 28.0 average 20 u, terminal & intercalary.

Oogonia 16.9 to 22.9 " 20.6 u.

Oospores 14.7 to 18.3 " 16.3 u.

Organism

Mycelium 3.0 to 7.0 u.

Conidia 18 to 25 u.

Oogonia 21.5 to 25.0 u, average 24 u.

Oospores 18.5 to 21.5 u, " 20.5

Although the cogonium is about the same size as that of P. de Baryanum, yet the oospore is much larger. However, if the two organisms were grown under similar conditions, there might not be so much difference.

Summary.

Radishes taken from various gardens in Madison in June, 1921, were affected with a black root disease. Radishes were replanted in two of these gardens later in the summer, but very few showed any symptoms of the disease.

Soil was taken into the greenhouse from one of these gardens and planted several times with radishes and sugar beets but nearly all plants were killed by damping-off in the seedling stage. This damping-off, however, may have been partially due to other organisms. One part of infected garden soil was mixed with three parts of ordinary greenhouse soil and planted with radishes. Some damping-off occurred and a few radishes develop-

ed black root.

An organism was isolated from damped-off sugar beets, grown in garden soil and the same organism isolated from damped-off radishes and also from the blackened area of a mature radish. As the organism was isolated, only once from the blackened area of a radish taken from garden soil, it is possible that other organisms may also cause this blackening.

In attempting to make isolations of an organism from the blackened area, one of the difficulties met with during the winter months, has been the obtaining of a sufficient number of diseased radishes with which to carry on such experiments.

When sterilized soil was inoculated and planted with radishes, the organism was isolated by plating out the radish seedlings on prune agar.

Radish and sugar beet seeds used in the experiments, where the soil was sterilized, were treated by heating in water at 60° C. for ten minutes on two consecutive days and then dried on filter paper immediately after heating.

When partially grown radishes were inoculated with the mycelium of the organism, they become diseased in a few days and when pieces of these were plated out on media, the organism was re-isolated.

The organism has been grown on various artificial media and on vegetable material in water plates with very similar results.

The fungus appears to belong to the genus Pythium and probably very closely related to Pythium de Baryanum Hesse.

In conclusion, the writer wishes to express his appreciation of the assistance given by Professor E. M. Gilbert during the studies of the organism and the preparation of this manuscript.

References.

1. Barrett, J. T. A serious root disease of radish. Phytopath. 2: 96. 1912.

2. deBary, A. Einige neue Saprolegnieen. Jahrb. Wiss. Bot. 2: 169-189; pl. 19-21. 1860.

3. Butler, E. J. An account of the genus Pythium and some Chytrideaceae. Memoirs, Dept. Agr. India. Bot. Series 1, no. 5: 1-160, pl. 1-10. 1907.

4. ----------Pythium de Baryanum Hesse. Memoirs, Dept. Agr. India. Bot. Series 5, no. 5:262-266, pl. 5. 1913.

5. Carpenter, C. W. Morphological studies of the Pythium-like fungi associated with root rot in Hawaii. Bul. Expt. Sta. Hawaiian Sugar Planters' Ass'n, Bot. Ser. 3, pt. 1: 59-65, pl. 16-23. (Reprint issued Aug., 1921).

6. Edson, H. A. Damping-off and root rot parasites of sugar beets. Phytopath. 3: 76, pl. 16-26. 1913.

7. ----------Seedling diseases of sugar beets and their relation to root rot and crown rot. Jour. Agr. Res. 4: 161-163, pl. 44+48. 1915.

8. ----------Rheosporangium aphanidermatus, a new genus and species of fungus parasitic on sugar beets and radishes. Jour. Agr. Res. 4: 279-291. 1915.

9. Miyake, K. Fertilization of Pythium de Baryanum. Ann. Bot. 15: 653-667, pl. 36. 1901.

10. Trow, A. H. Observations on the biology and cytology of Pythium ultimum, n. sp. Ann. Bot. 15: 269-312, pl. 15-16. 1901.

11. Willis, M. A. Black root of radish. M. S. thesis, Univ. of Wisconsin, 1914.

Appendix.

The following are some of the media which have been used in making a study of the organism.

String-bean agar.

String-beans	400.	gms.
Agar	17.5	"
Distilled water	1000.	C.C.

Cut up the pods, using only clear healthy ones, and cook for two hours in a steamer in 500 C.C. of the water. Dissolve agar in 500 C.C. of the water in steamer. Strain off the beans, make the amount of bean water up to 500 C.C. and add it to the agar. Mix thoroughly and heat for a short time, then filter, tube and autoclave at 7 to 10 lbs. pressure for 30 min.

Cornmeal agar.

Cornmeal	35 to 40 gms.
Agar	30 "
Distilled water	1000 C.C.

Heat the cornmeal in 500 C.C. of the water at 60° for one hour. Dissolve agar in 500 C.C. of the water in the steamer. Strain the cornmeal through cheese cloth and add sufficient water to cornmeal water to bring it up to 560 C.C., then add it to the agar. Mix and heat for a few minutes, then filter, tube and autoclave at 7 to 10 lbs. pressure for 30 min.

Prune agar.

Sweet prunes	40 to 50 gms. (without pits)
Agar	30 "
Distilled water	1000 C.C.

Cook prunes for one hour in 500 C. C. of the water. Dissolve agar in 500 C. C. of the water in the steamer. Strain off prunes and add prune water to agar. Mix thoroughly and heat 10 to 15 min. Filter, tube and autoclave at 7 to 10 lbs. pressure for 30 min.

The following illustrations have been made with the camera-lucida, x 715.

1. Germinating conidia observed after organism had been in hanging drop for one week. No further change took place although observed closely for forty-eight hours.

2. Antheridium and oogonium as they may be seen in a hanging drop at the time of fertilization.

(a) before fertilization.

(b) after fertilization.

a and b are not the same structures.

3. Fertilization taking place where the antheridium comes from another hypha.

(a) before fertilization.

(b) after fertilization.

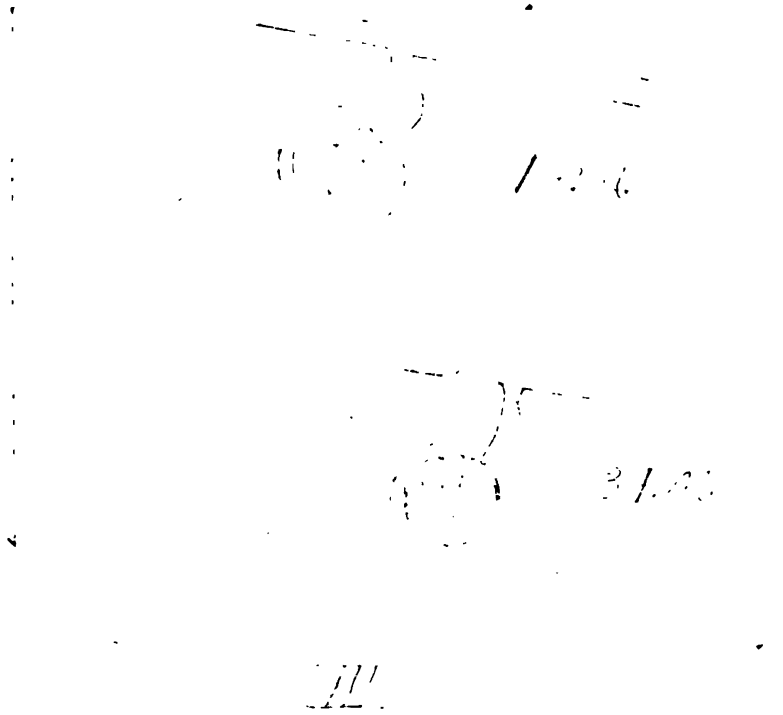
4. Oogonium with two antheridia, - observed in a drop of string bean agar four days after it had been inoculated with a small portion of the mycelium.

5. Fertilization and development of oospore, three days after mycelium had been placed in hanging drop.

(a) 3 P.M. (b) 8 P.M. (c) 8 A.M.

6. Oospore and oogonial wall, twelve days after mycelium had been placed in hanging drop.

I



Approved Ellis Gilbert

May 11, 1922